A RAPID STAINING APPARATUS.

C. M. MIX, ITHACA, N. Y.

Methods of staining may be roughly arranged in three classes: staining in toto, staining the sections and carrying them through all the steps necessary previous to mounting them in balsam on the slides, and, finally, performing all the work of staining after the sections have been fastened to the slides.

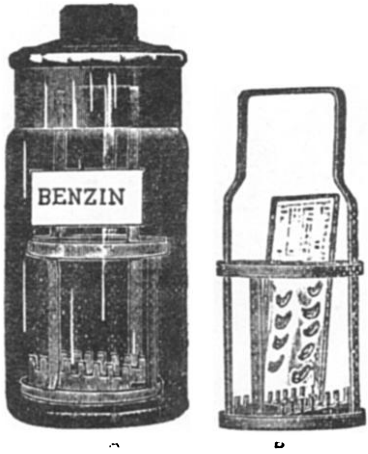


Fig. 1.

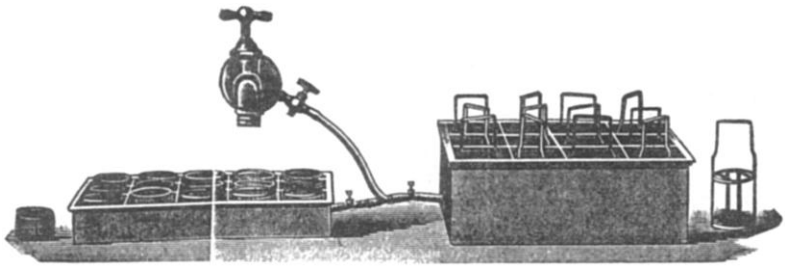
The first method is an excellent one, when small pieces of tissue are used. Large pieces would not be penetrated evenly by the staining agent. This method is very rapid; for the sections can be mounted directly from the knife in Canada balsam after removing the paraffin, or, in case the object is imbedded in collodion, it is only necessary to remove the oil, dehydrate, clear and mount. On account of the difficulties in securing good penetration in the staining fluids, this very efficient method has, we are loath to note, a rather limited application.

In most cases better results are obtained by staining after the sections are cut. As was suggested above, this result may be obtained in two ways. When pieces of firm, homogeneous tissue, such as pieces of liver, are employed, and in case it is not necessary to preserve the continuity of the series of sections, good results may be obtained by placing the sections as

soon as cut in watch glasses, filled with the proper reagents. By transporting them from one dish to another by means of a section lifter, or even by means of a glass rod, the sections may be carried through the various processes necessary to prepare them for mounting, before they are placed upon the slide at all.

This method is entirely inapplicable to serial work, as in embryological investigations for instance. Only firm structures could be treated in this way, for the more delicate ones would rapidly go to pieces, after the removal of the paraffin, without something to hold them in place. Even firm tissues are in great danger of being torn and distorted, or entirely destroyed, by being so often handled. Thus it appears that, for work in which delicate structures are involved, or for pieces of considerable size, both the above methods fail.

To meet these difficulties, the method of staining on the slide



A Fig. 2. B

has been resorted to. As applied in the laboratories of Cornell University, the method is as follows: the sections are fastened to the slide by means of a thin coat of albumen and heat, if imbedded in paraffin, or by a drop of ether-alcohol, if collodion is used. After the removal of the paraffin or oil by means of benzene or xylene, they are treated with ninety-five per cent alcohol. They are now ready to be stained. The slides may now be placed in either the ordinary Stender dish, containing the staining agent, or laid flat on the rack (r, Fig. 5) over the waste jar (w, Fis. 5). In the latter case, the staining agents are poured upon the slides by means of pipettes. Excellent results are uniformly obtained in this way, in serial as well as

single sections. Since the section is firmly fastened to the slide, the relative position of the different parts of the tissue is not changed, and the section does not become broken, or lost. If several slides are placed in one of the Stender dishes at the same time, there is always danger of hitting them together and thus destroying the sections. This difficulty becomes particularly annoying in serial sectioning, where, of course, it is of the utmost importance to preserve every section intact.

To hasten the process of staining on the slide and to reduce the danger of injury to the sections, the apparatus described below has been devised. By means of this device, fourteen

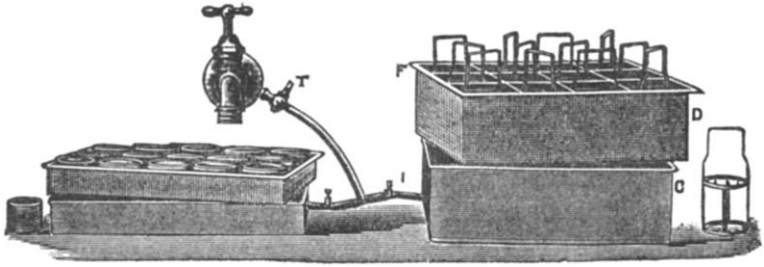


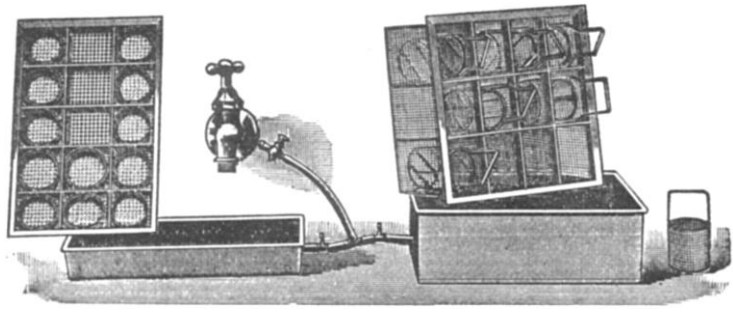
Fig. 3.

slides can be stained in the time usually required for one, and the danger of injury to the section is entirely obviated. This apparatus was designed and its efficiency thoroughly tested in the laboratories of Cornell University.

The apparatus was designed primarily for work with Heidenhain's iron-hematoxylin, in the use of which, in order to obtain a permanent stain, it is necessary to wash the sections for some time in running water. Hence, with the essential part of the apparatus, there is combined a washer, which will be described later. The principal part of this staining device is a carrier, or slide holder (Fig. 1, b). It consists of two rings cut out of stiff sheet brass. The rings are about one-third of a centimeter in width and about five centimeters in diameter. They are held parallel to each other and about six centimeters apart by four upright standard pieces of the same material.

These upright pieces are arranged parallel to each other and at right angles to the rings. Two of them extend about six centimeters above the upper ring to form the handle. In this way we have a skeleton basket. Across the bottom ring extend two parallel pieces of brass, arranged at right angles to the handle. In the upper edge of each of these cross strips are seven notches, opposite each other, and of such a size as to receive, in each pair of notches, the ends of two slides, placed back to back (Fig. 1, a and b). These carriers are five centimeters in diameter and hold fourteen slides. They are made to fit a museum jar of convenient size, described above (Fig. 1, a). Any vessel of convenient size might be used with carrier to match.

This jar for holding the reagents is the No. 2605 made by Whitall, Tatum & Co., New York City. It is listed in their catalogue as museum jar—diameter two inches; height to shoulder, three and three-fourths inches; height to top of stopper, five and one-half inches; width of mouth, two inches.



A Fig. 4. B

The handle of the carrier extends into the hollow stopper when the vessel is closed. These glass stoppers are ground to fit the necks of the bottles, so that the vessels are tightly closed, and in consequence evaporation is prevented.

The third part of this apparatus consists of a washer very similar in construction to and identical in principle with the tissue washer described by Prof. Gage in his article in the July (1898) Journal of Applied Microscopy (Figs.

2, 3 and 4, b). The washer consists of two parts—an oblong brass box 24 centimeters long, 19 centimeters wide, and 9 centimeters deep (Fig. 3, c). At one of the lower corners is an inlet tube (i) to which is attached a piece of rubber tubing extending to the tap (t) from which is derived the supply of water. Inside this box (c), which is water tight, is a second box (d), made one centimeter smaller all around, so as to easily fit inside of the first. From the upper edges of this inside box there projects a flange (f) which rests upon the upper edges of the outside box. Thus a water space of about one centimeter is left between the outer and the inner box. The inner box, unlike the outer one, is made of perforated brass and allows the water to pass freely through it. By means of five cross partitions, which intersect at right angles, the perforated box is divided into twelve compartments, each six centimeters square (Fig. 2, c). Each compartment is large enough to hold one of the slide carriers. In this way a constant and gentle current is maintained, and the preparations do not become dislodged from the slides.

Fig. 5.

The slides with the preparations attached are placed in the notches back to back. Then the carrier with its fourteen slides is placed successively in the various reagents contained in the jars described above. When hematoxylin and some counterstain, as picro-fuchsin, are used, six jars are necessary to complete the outfit (Fig. 5).

The advantages of this apparatus over the old method are obvious at a glance. The slides are not touched, either with fingers or forceps, from the time they are placed in the carrier until they are removed from the clearer to be mounted. They are held in a stable position, so that it is impossible for the preparations to be injured by hitting against each other or the sides of the jar. By exercising a little care in lifting the carrier from the liquid, only the gentlest of currents is produced. In the hands even of an unskilled operator, the danger of injury to the sections is reduced almost to zero. Fourteen slides can be prepared with the labor incident upon the preparation of one by the old method. When a large number of slides is being prepared, it expedites matters to start a second carrier of slides as soon as the first carrier is removed from the first bottle, and so on until the whole number to be prepared is under way. This applies especially to serial work or the making of large numbers of duplicate slides for classes.

In a word, this apparatus, which, in its simplest form, need consist only of the carrier and the reagent jar, simplifies and makes available for wholesale preparation the best and most accurate method of staining, namely, the method of staining on the slide. It removes all danger of accident to the sections. The danger of distortion is reduced to a minimum. Great rapidity is obtained, and a complicated process is simplified.

Cornell University.